FECUNDATION AND FORMATION OF THE PRIMARY ENDOSPERM NUCLEUS IN CERTAIN LILIACEAE

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(WITH PLATES III–V)

Introduction

Between 1890 and 1902 many articles appeared on fecundation and double fertilization in the angiosperms. On the whole, the authors have dealt with the entrance of the male nuclei, their behavior within the embryo sac, their form, the union between the egg and male nucleus, and between the male and polar nuclei; but they have not investigated in detail the chromatin changes that occur from the time of contact of these nuclei to the completion of the first division.

In 1891 GUIGNARD (6) described the entrance of the so-called antherozoids into the sac, each accompanied by its centrosomes. One male nucleus became applied to the egg nucleus, each of which took on the resting condition and remained distinct for some time. While in this state the male nucleus enlarged and both the egg and the sperm nucleus flattened at the surface of contact, but with a distinct line of demarcation remaining between them for some time. Even after the nuclear membranes had disappeared, the contour of the two was traceable at the periphery. Later he distinguished, on opposite sides of the nuclear cavity, two groups of chromatin in the spirem stage. No drawings were made to show this. When the nuclear plate was formed, he asserted that one-half of the chromosomes were contributed by the egg and one-half by the sperm.

The process of fertilization in *Lilium Martagon* and *L. candidum* was described by MOTTIER (13) in 1898. In *L. Martagon* there was no complete fusion of egg and S-shaped sperm, the lack of which resulted in a failure to mature seeds. In the region of the two polar nuclei, which had not fused and which began disintegrating 96 hours after pollination, a nucleus, similar to the nucleus which united with

the egg, was observed. *L. candidum* furnished material for normal fertilization. At the union of the egg and sperm, the latter was about the size of the former and both were in the resting condition, the chromatin being distributed in the form of a fine network. No boundary was observed separating the two elements at the point of contact, and the fusion that took place during resting condition was so complete at the close of fertilization that there was no visible distinction between male and female chromatin.

In 1904 MOTTIER (14) confirmed his earlier investigations, pointed out the S shape of the male nucleus, the fusion of the sexual nuclei in resting condition, the coming together of the two polar and male nuclei in *L. Martagon*, and the cause of the non-fusion. Although he stated that the sexual nuclei were in the resting condition at the time of fusion, he called attention to the chromatin of the sperm being more regular than that of the egg. It was also claimed that the nucleoli fused at fertilization.

One of the first reports of double fertilization was made by GUIGNARD (7) in 1899 for *Lilium Martagon*. In this species he observed the union of one of the male nuclei with one of the polar nuclei, followed by the union with the second polar nucleus. The chromatin of the two male nuclei, on account of being coarser, was distinguishable from that of the egg and polar nuclei with which they had fused. He also stated that he was able to recognize the triple origin of the secondary nucleus during the prophases, although no drawings were given.

NAWASCHIN (15), in the first report of double fertilization in *Lilium Martagon* and *Fritillaria tenella*, noted that the cellulose membrane surrounding the sexual apparatus was absorbed just previous to the entrance of the pollen tube, and that the spiral-shaped male nucleus entered the protoplasm of the sac. He concluded that the sperms took on various shapes under various conditions, and, as GUIGNARD had assumed, that they were motile. One sperm was found to enter the egg, the other to unite with the superior polar nucleus, and in both cases a complete fusion occurred after a certain period. The fusion of the superior and inferior polar nuclei took place after the male nucleus had united with the former. The triple fusion was followed in a short time by a division which preceded that of the egg.

In 1900 GUIGNARD (**9**) found that in some cases the polar nuclei, the upper one of which he said was analogous to the egg, fused before the entrance of the pollen tube, and that when the male nuclei entered the sac, they entered into fusion so quickly that, in some species, one rarely saw them free. In *Tulipa* he was able to follow the contour of the three nuclei entering into the fusion nucleus some time after their coming together, and even after the membranes had disappeared at their surface of contact.

As a result of investigations in various groups of angiosperms by GUIGNARD (**6, 7, 8, 9, 10**), NAWASCHIN (**15**), STRASBURGER (**17, 18**), and others at this time, it was generally concluded that double fecundation was normally found in angiosperms and that the uniting of the male nucleus with the polar nuclei was in the nature of a pseudo-fecundation whose function was to stimulate the formation of "albumen."

ERNST (**3**), investigating fertilization of *Paris quadrifolia* and *Trillium grandiflorum*, found that the two polar nuclei were fused before the male nucleus united with them, and at times a spirem was formed previous to the entrance of the sperm, showing that the male nucleus was not necessary to stimulate division. At other times the spirem was not formed until the three nuclei were fused, in which case he was unable to discern which part of the chromatin was contributed by the various nuclei. He also stated that it was not safe to rely upon the number of nucleoli found in the fused mass to ascertain whether fertilization had taken place or not. In the fecundation of the egg there was a complete blending of the substances, and at cross segmentation he failed to find the arrangement of the chromatin into two groups.

STRASBURGER (**17, 18**) used the terms generative and vegetative fertilization, the latter being applied to the triple fusion. The union of the sperm, either with the egg or with the polar nuclei, functioned as a stimulus.

In 1911 COULTER (**2**), after reviewing the literature on endosperm formation, stated that since endosperm may form without the fusion of the sperm or even of the second polar nucleus, these being simply supplementary, there seemed to be no reason why "there should be any hesitation in recognizing the endosperm as gametophyte." He concluded that "the product of such fusions is

merely an undifferentiated tissue which practically continues the tissue of the gametophyte, that is, it is simply growth and not organization."

From 1902 to 1913 practically nothing new was published on the subject of fertilization. In 1913 BLACKMAN and WELSFORD (1) reported that the chromatin of the vermiform male nucleus was in a network, although not the network of a resting nucleus, this condition becoming more noticeable later on. At times they also noted that the chromatin of the egg might become threadlike just previous to fusion.

The most recent paper on fertilization is by SAX (16) in 1916 on fertilization in *Fritillaria pudica*, in which he noted that the vermiform sperm lay indented in the egg for some time before the membranes between them disappeared. The chromatin was in more or less of a network and the granules were of various sizes. When the membranes at the surface of contact broke down, the contents of the two nuclei mingled and were not distinguishable from each other. The spirem usually appears after this. Triple fusion was also complete and the resulting nucleus divided before that of the fertilized egg.

In none of these cases have the chromatin changes been carefully followed from the time of contact of the nuclei until the completion of the first division, the emphasis previously having been placed upon the actual coming together, the uniting, and the very earliest steps in division.

The process of fertilization and distribution of the chromatin contributed by the egg and sperm in *Pinus* and *Abies* has been carefully worked out by FERGUSON (4, 5) and HUTCHINSON (12), and it was with the desire that something of this nature should be done for angiosperms that the present investigation was undertaken.

Materials and methods

For fertilization of the egg, *Trillium grandiflorum* was used, the material being collected in damp woods along the Des Plaines River, northwest of Evanston, Illinois, from May 3 to May 26, 1916. The first collection was made at the time of pollination, although the pollen tube was not seen in the micropyle until two weeks later,

while in the collection of May 26 dividing endosperm nuclei and second division of the fertilized egg were found for the first time. *Lilium Martagon*, collected from the garden of Indiana University, Bloomington, Indiana, in May 1916, 96 and 120 hours after pollination, was more favorable for the first division of the endosperm nucleus. The former material was killed and fixed in chrom-osmic-acetic acid 1–2 hours and then in chromo-acetic acid 24–36 hours, washed, dehydrated, and imbedded either from chloroform or xylol. *Lilium Martagon* ovaries were killed and fixed in chrom-osmic-acetic acid 24–36 hours, washed, dehydrated, and imbedded from chloroform. All sections were cut 12 μ thick and both modified triple and Heidenhain's iron-alum-haematoxylin were used for staining, the latter being more satisfactory for most stages, as the chromatin was more sharply differentiated.

Formation of the primary endosperm nucleus

For the development of the spirem and the first division of the endosperm nucleus, *Lilium Martagon* was found to be very favorable, as many dividing primary endosperm nuclei were found in the sacs of material killed 96 and 120 hours after pollination. Activity did not cease at the end of the first divisions, for as many as 12–16 nuclei were found in many sacs of the older material (fig. 29).

The sperm comes in contact with the polar nuclei before these two have fused, although they may be in contact or in close proximity (fig. 17). These three nuclei will usually be found in the center of the sac where just previous to the triple fusion the two polar nuclei were to be seen.

The chromatin of the egg can scarcely be said to be in a network, but rather to consist of strands which are more or less united (figs. 17, 18), that of the male nucleus being much coarser than that of the polar nuclei. When the sperm reaches the middle of the sac, it still has its curved or vermiform shape, while the contour of the polar nuclei may vary, sometimes being quite curved before coming together (fig. 17), but at other times only changing to this shape as pressure is exerted by contact. The three nuclei upon uniting may be variously twisted about each other, the male nucleus usually twisting more than the others and recognizable by

its coarser chromatin strands (fig. 18). In fig. 18 the three nuclei as a whole present a more or less globular contour, although the nuclear membranes are still present at the surfaces of contact.

In *Trillium grandiflorum* the three nuclei, which unite to form the primary endosperm nucleus, are all alike in shape, it being impossible to distinguish the male nucleus from the two polar nuclei by its form or size (fig. 16). Since the mass of the three nuclei is so large, it is often impossible to find parts of all three nuclei in one section, and frequently only two will be visible (fig. 19). All three contain nucleoli, sometimes one, while at other times there are many. The chromatin strands thicken until they may be traced for a considerable distance (figs. 19, 20). While in some instances the membranes still separate the nuclei (fig. 20), at other times they are not visible, as in fig. 19; but, nevertheless, where the chromatin contributed by one nucleus leaves off and that of another begins is very easily seen.

Up to the period when the spirem has assumed its mature thickness, the separating membranes may not have entirely disappeared (fig. 21), and in some cases the three groups of spirems are plainly evident. From fig. 22 it could be concluded that a complete fusion or intermingling of chromatin had previously occurred, but such has not happened, for in the next section of this same primary nucleus parts of all three nuclei are seen (fig. 21). Even at this stage, before the complete breaking down of the separating membranes, segmentation has begun and spindle fibers are forming about the group. As far back as the coming together of the two polar nuclei and the sperm nucleus, a surrounding complex of fibers could be seen (fig. 18).

In fig. 21 the fibers have commenced to radiate out into the cytoplasm, followed after a short period by a complete segmentation of the spirems, resulting in three groups of chromosomes being scattered upon the three arms of the tripolar spindle, respectively (figs. 23, 24). As the tripolar structure gradually assumes the form of a bipolar spindle, the chromosomes, which were previously lying upon the third arm, are pulled into line with the other two groups, thereby forming a typical bipolar spindle (figs. 24, 25). The chromosomes now thicken and are typically arranged into the

equatorial plate of the bipolar spindle; but even yet the third group of chromosomes is recognizable, as can be seen at the left in the group in fig. 25.

After this stage the chromosomes contributed by each of the polar nuclei and by the sperm nucleus are no longer distinguishable (fig. 26). No trace of such distinction is seen in early metaphase or later spindle phases. How the various chromosomes finally arrange themselves upon the spindle and their distribution could not be ascertained in this investigation. When the $3x$ chromosomes have gathered upon the equatorial plate of the bipolar spindle, each very much elongated chromosome splits longitudinally (fig. 26) preparatory to a typical equational division. No intermediate stages between early metaphase and early telophase were found. As the chromosomes reach the poles, they are somewhat shorter than when leaving the equator, and from the count, as seen in fig. 27, $3x$ chromosomes have passed to each pole.

In the third division of the endosperm nuclei of *Trillium grandiflorum* a peculiarity was noted. In one of the dividing nuclei there were still to be seen the three groups of chromosomes upon the spindle, each group consisting approximately of six chromosomes, or the haploid number. It is easily seen that there is a great similarity in appearance between this third division of the endosperm and the first division of the primary endosperm nucleus. A similar stage was observed in the second division of the endosperm nucleus of *Lilium Martagon* (fig. 29), showing in the upper dividing nucleus an appearance very similar to that seen in fig. 24. It was not determined how long endosperm division would continue in *Lilium Martagon*, as nothing older than 120 hours after pollination was collected.

Fertilization of the egg and its first division

Trillium grandiflorum furnished the best material for this phase of the investigation, as the later stages of the first division of the fertilized egg were not to be found in *Lilium Martagon* collected 120 hours after pollination.

In *L. Martagon* the chromatin of the egg and the sperm, at the time when the male nucleus lies coiled upon the egg, is similar in

appearance to that described for the polar nuclei and the second sperm nucleus. The chromatin is in strands, that of the sperm being heavier than that of the egg (fig. 1). The sperm fertilizing the egg is very much smaller than the sperm uniting with the polar nuclei at the time of contact and not so vermiform (compare figs. 1 and 17). Fig. 2 illustrates a typical fertilized egg of *Trillium grandiflorum* just a little later in development than that of *Lilium* (fig. 1). In this later stage the chromatin is lumpy, the particles being larger in the sperm than in the egg, and the membranes separating the two nuclei are becoming very thin, so that it is difficult to distinguish them at all times. After this time these membranes are rarely to be found, although in some instances they persist for a longer period (fig. 4).

The chromatin gradually collects into larger groups, forming more or less broken threads connected with each other by fine anastomoses (figs. 3, 4). In many portions of the nucleus of this fertilized egg the parallel nature of some of these strands is quite conspicuous (figs. 3, 4). In some fertilized eggs, as for example in fig. 5, a more or less beaded, although discontinuous, spirem was noted. Even though the nuclear membranes which separated the egg and the sperm have disappeared, the chromatin that has been contributed by each of the two nuclei remains distinct (fig. 3). This condition is much more evident in some fertilized eggs than in others. The sperm at the period of union contains a much smaller amount of chromatin than the egg and throughout most of the subsequent stages this condition persists (figs. 3, 4, 9, 10, 12). During all this time the fertilized egg is growing in size and increasing the amount of chromatin. When the continuous spirem is first formed, it is quite thin (fig. 6), but as the prophase advances the chromatin thread thickens and shortens until a comparatively thick spirem results (figs. 6–12). Instead of one continuous spirem, two distinct spirems are usually to be seen within the single nuclear cavity, although located in different parts of the cavity (figs. 8–10). In some sections such differentiation is not visible (figs. 6, 7). The dotted lines *a–b* in figs. 9 and 10 separate the two spirems, one of which was contributed by the sperm, the other by the egg.

In spite of the fact that a nucleolus is not seen in the sperm when it unites with the egg, very small ones are found in later

stages (fig. 3), and still later, in the spirem stage, a large nucleolus is frequently observed (fig. 9); but at the beginning of segmentation all traces of these nucleoli have vanished.

With the beginning of segmentation, the chromatin threads appear to contract, presenting the appearance of "the second contraction" of heterotypic mitosis (figs. 11, 12). Following this, the nuclear membrane surrounding the two groups disappears, leaving the massed segments lying free in the cytoplasm. Even now the two sets of chromosomes are separate (fig. 12), and to all appearances a spindle is formed about one group of chromosomes and the other set is pulled into the bipolar spindle, for, as late as in fig. 13, the chromosomes contributed by the sperm are distinct from those contributed by the egg. In each of the two groups (fig. 13) there are approximately six chromosomes, or the haploid number. The writer was unable to determine the arrangement of these two sets upon the equatorial plate, owing to lack of material for later stages. From the number of chromosomes seen at telophase and later divisions, each splits longitudinally at metaphase, so that twelve, the diploid number, pass to each pole.

Discussion

A very full, detailed account of fertilization in *Pinus* by FERGUSON and in *Abies* by HUTCHINSON has been published; but a similar account is not to be found for angiosperms.

FERGUSON (5) reports that in *Pinus* the chromatin of the egg is arranged in an interrupted reticulum, the network consisting of granules of various sizes in a colorless linin. When the contents of the pollen tube have been discharged into the egg, one of the male nuclei takes up a position on the concave side of the egg, this depression having been formed at the approach of the male nucleus. Gradually from each nucleus a spirem is formed from the respective chromatin material, at which period fibers arise in the region of the spirems and the nuclear membrane gradually fades away. At segmentation these two spirems give rise to two groups of chromosomes, but as they collect on the spindle this distinction is lost. Each chromosome splits longitudinally and each daughter nucleus receives the diploid number. When these daughter nuclei are preparing for second division, the chromatin collects into two

spirems, the steps being very similar to those of the first division, and it is concluded that in all probability they come from the maternal and paternal source respectively, in spite of the fact that in the formation of the daughter nucleus the chromatin has appeared completely fused. Since the subsequent divisions were not followed, it could not be determined how long this dual nature persisted.

The account of fertilization in *Abies balsamea* by HUTCHINSON (12) varies somewhat from that of FERGUSON. The contents of the male nucleus pass into the nucleus of the egg, although the chromatin groups remain distinct, and later, when the two sets of spindle fibers are formed, two sets of chromosomes arise from the respective nuclei. These two spindle complexes unite and the chromosomes of the maternal parent pair with the chromosomes of the paternal parent, after which the fibers disappear. The members of each pair twist about each other, bend, and become transversely segmented at the bend so that there are $2x$ pairs in the fertilized egg. When the second set of fibers appears, the members of the pairs resulting from the transverse segmentation separate for the opposite poles.

HEATLEY (11) has described the development of the embryo sac of *Trillium cernuum*, in which the sac arises from the chalazal daughter nucleus of the megaspore mother cell, two megaspores only being functional. Each functioning megaspore divides twice to form the typically arranged 8-nucleate embryo sac.

In the present study it was not considered necessary to work out the development of the sac, and furthermore, no attempt has been made to determine the method of entrance of the sperms into the sac or their passage to the egg and polar nuclei. BLACKMAN and WELSFORD (1), ERNST (3), GUIGNARD (6, 9, 10), MOTTIER (13, 14), SAX (16), and STRASBURGER (17, 18) have reported on the earlier phase of fertilization, and on the whole have agreed. These same authors have also described in detail the coming together of the nuclei, their chromatin condition, and the breaking down of the nuclear membranes separating them, although GUIGNARD (7–9) differs somewhat from the others on the latter point, which will be spoken of later. By comparing these investigations with those of *Pinus* (FERGUSON 4, 5) and *Abies* (HUTCHINSON 12), it is apparent

to the writer that the present knowledge of certain phases of fertilization in angiosperms is very scanty, especially as to the fate of the maternal and paternal chromatin.

ERNST (3) reports for *Trillium grandiflorum*, and a similar conclusion is reached by various authors for certain other plants, that there is a fusion of the polar nuclei previous to the entrance of the pollen tube; but in not a single case in either *Trillium* or *Lilium* has such a condition been found to exist, the polar nuclei always being distinctly separate, although usually in contact (figs. 16–19). Only a few cases of triple fusion were observed in *Trillium grandiflorum*, although all that were found appeared as illustrated in fig. 16.

BLACKMAN and WELSFORD (1), GUIGNARD (7–9), MOTTIER (14), and SAX (16) have noted that the male nucleus can be distinguished from the egg and from the polar nuclei both by its shape and the condition of the chromatin, since this substance is coarser in the male nucleus, and at times assumes almost a spirem condition previous to fusion. The sperms have been found not always to retain their S or curved form, for in *Trillium* the male nucleus could not be distinguished from the polar nuclei, either by its size or countour (fig. 16).

The three nuclei (superior and inferior polar nuclei and male nucleus) of *Lilium Martagon* become very much twisted about each other very soon after coming in contact (fig. 18), and even previous to this the polar nuclei may have lost their globular form (fig. 17), although the writer failed to find any mention of this in previous accounts. The fibers appear early about the nuclear complex of *Lilium* and gradually merge into the cytoplasm, as FERGUSON (4, 5) has reported for the fertilized egg of *Pinus*. The chromatin is in fine strands and not in a network, as GUIGNARD and MOTTIER have stated. The number of nucleoli in each nucleus may vary from one to several, and in some specimens there are none. As SAX (16) has said for *Fritillaria*, at the time of contact the chromatin is threadlike, with large irregular pieces of chromatin scattered throughout.

In many instances the separating nuclear membranes are still to be seen when the chromatin has been transformed into a

comparatively heavy spirem (fig. 20), and in some instances at the beginning of segmentation fragments of it still remain (fig. 21). In cases where the separating nuclear membranes do disappear early, the limits of the nuclei are readily followed (fig. 19). GUIGNARD (7), in his first report of double fecundation, says that the chromatin of the sperm enters into more or less of a spirem before fusion with the two polar nuclei, after which, at times, he is still able to recognize the triple origin of the secondary nucleus of the sac. None of his drawings are later than fig. 18 of the writer, and apparently the chromatin is in the same condition. In a later paper (8) he describes a similar condition in *Narcissus*.

In *Fritillaria*, SAX (16), after stating that the chromatin of the male nucleus frequently passes into a spirem previous to the breaking down of the separating membranes, and in some few instances observing the beginning spirem in the polar nuclei, concludes that there is a complete fusion of the chromatin contributed by the three nuclei, and that this is further proved by finding no incomplete fusions in later stages.

In not a single specimen showing the formation of the primary endosperm nucleus was the writer unable to distinguish between the chromatin of the various nuclei that have contributed to this nuclear complex. From the view obtained in fig. 22 it could readily be concluded that a complete intermingling of chromatin has previously occurred, but when the next section of the same primary endosperm nucleus is examined (fig. 21), such a conclusion is seen to be groundless.

Whether or not the separating nuclear membranes have entirely broken down by the time of segmentation, the spirems remain distinct, and, following segmentation, three groups of chromosomes collect upon the three arms of the tripolar spindle (figs. 23, 24). In none of the literature examined has such a stage been shown or reported, for, if such had, the idea of complete fusion or intermingling of chromatin material could not have been adhered to up to the present time.

Since the chromosomes are very long and quite numerous (36), the writer was unable to follow definitely their final arrangement upon the bipolar spindle. From the appearance of fig. 25 it seems

that the group on the right side of the equatorial plate might be the group that has been pulled into line. Soon after this each chromosome splits longitudinally, as FERGUSON (**4, 5**) has reported for *Pinus*, and all trace of the individuality of the groups is lost for a time.

It has been generally understood that in *Lilium Martagon* fusion and subsequent divisions did not occur unless the top was cut off from the bulb; but in the plants used in this investigation, in which this was not done, the ovaries showed many dividing endosperm nuclei and the sacs were in good condition (fig. 29). In *Trillium grandiflorum* in a sac of four dividing endosperm nuclei (fig. 28), and in *Lilium Martagon* in a sac of two dividing nuclei (fig. 29), as described previously, three groups of chromosomes are seen on the spindle. This corresponds to the condition of the second division of the oosphere, as FERGUSON (**4, 5**) has reported for *Pinus*, in which she notes that the second division is like the first, there being two spirems.

Figs. 28 and 29 distinctly show the three groups, and if such a condition is normal the question arises whether the male and female chromatin remaining distinct is the cause of the mottled appearance of some hybrid endosperms as found in *Zea Mays*. As has been observed by many investigators upon chromosome count in endosperm when it consists of many nuclei, the number varies in the different nuclei, there no longer being the $3x$ number. If in some of the divisions, when there is not an equal distribution of chromosomes, which is common in endosperm divisions, one group should pass to one pole and two to the other, the chromatin brought in by the sperm would then be in one nucleus by itself, or with one of the polar nuclei, thus causing the mottled appearance in the endosperm as seen in *Zea Mays*.

The earlier writers on double fertilization, STRASBURGER (**17, 18**), MOTTIER (**14**), NAWASCHIN (**15**), and ERNST (**3**), and the latest investigator SAX (**16**), concluded that there was an intermingling or a complete fusion of the chromatin contributed by the sperm and two polar nuclei in the formation of the primary endosperm nucleus, and ERNST (**3**) further stated that he was unable to recognize at segmentation or in spirem the chromatin

that had been contributed by the respective nuclei; but the writer, because of the numerous specimens showing the distinct spirems, the three groups of segments, and the groups on the tripolar spindle, is unable to accept these conclusions for the primary endosperm nucleus of *Trillium grandiflorum* and *Lilium Martagon*.

What has been said for the chromatin of the primary endosperm nucleus of *Lilium Martagon* applies for the most part to that of the fertilized egg of *Trillium grandiflorum* and the early stages of *Lilium Martagon* and *L. philadelphicum*.

After a careful investigation of *L. Martagon* and *L. candidum*, MOTTIER (13) reported in 1898 that there is a complete fusion of the male nucleus and the egg nucleus in the resting condition, the chromatin being in a fine network. If the subsequent steps are not followed out, such an interpretation could be made for *Trillium*. Figs. 1 and 2 show fertilized eggs in which the sperm is lying coiled upon the egg, the male chromatin material being in coarser strands than in the egg. In some instances the nuclear membranes separating the nuclei break down early (fig. 3), while in others they persist for some time (fig. 4). It was the appearance of such stages as fig. 3, and some that will be spoken of later, that caused previous investigators (MOTTIER, NAWASCHIN, SAX, STRASBURGER, and others) to conclude that there was a fusion to the extent that the individual components were not recognized.

From sections showing the spirems (figs. 6–11) it appears at first sight that the interpretations of figs. 9 and 10 would be different from those of figs. 6 and 7; for in figs. 9 and 10 two spirems stand out distinctly, while in the other two there appears to be only one. If the reader will consider all the various angles from which the fertilized egg might be cut and all the various positions the two spirems might occupy within the cavity, it will be apparent that frequently the sections might be so cut that the dual nature of the spirems would not be seen. The significance of the contraction, similar to the second contraction of the heterotypic mitosis that occurs just previous to or during segmentation (figs. 11, 12), the writer is unable to interpret. There was no tripolar spindle observed in the fertilized egg, and from the appearance of fig. 13 it seems that only a bipolar spindle is formed and the second group

of chromosomes is pulled in upon it. In each group there are approximately six, the haploid number.

In 1891 GUIGNARD (6) pointed out in *Lilium Martagon* that there were two spirems and that one-half of the chromosomes on the spindle were contributed by the male parent and one-half by the female parent. No drawings were made to substantiate these views, and ERNST (3) and MOTTIER (13) apparently made it so conclusive that there was a complete fusion or intermingling of chromatin that GUIGNARD's earlier views were discarded and practically forgotten. In later papers GUIGNARD himself did not place much emphasis upon these earlier views.

SAX (16) stated that a spirem was frequently found in the egg and sperm before fusion occurred (fig. 21), but says "the rare appearance of such cases as that of the spirem stage in the egg and male nuclei when their outlines are still distinct, is probably of little significance in this respect. It is probable that these nuclei subsequently fuse completely, because no later stage of incomplete fusion was found."

Many writers have looked upon the number of nucleoli present in the fertilized egg as an indication that fertilization has or has not occurred. This, as ERNST (3) has pointed out, is not a safe indicator, for, as shown in figs. 5, 6, 9, 19–23, the nuclei have already united and from two to many nucleoli are present.

SAX (16) says "fig. 19 shows a stage where the common boundary has disappeared, the contents apparently mingled, and those from the male and female nuclei are not to be distinguished." In case of the formation of the fertilized egg, as in the formation of the primary endosperm nucleus, the writer is unable to agree with SAX and the earlier writers that there is a complete fusion or intermingling of the chromatin of the egg and the sperm; the material so plainly shows that the two remain separate from the time of coming together until the formation of the daughter nuclei. What happens after that does not come within the scope of this paper.

Conclusions

From the stages that have been found in the fertilized egg leading up to the first division, and in the primary endosperm nucleus up to

the time of the third division of the endosperm, it is evident that they are analogous to those of *Pinus*, as reported by FERGUSON (**4, 5**), although nothing was observed that would correspond to those steps in fertilization, as reported by HUTCHINSON (**12**) for *Abies*, which differed from those of *Pinus*.

To the writer the finding of the separate, distinct spirems and the separate groups of chromosomes is added evidence that the chromosomes maintain their individuality from one generation to the next.

In conclusion the writer wishes to state that, according to her interpretation of the word "fusion" as used by previous writers, there was meant a mingling of the male and female chromatin, so that all trace of the individuality of chromatin and chromosomes contributed by the respective parents was lost by the time of the first division. In this investigation no such fusion was found, but instead, an entrance of two or three masses of chromatin, as the case might be, into a more or less single nuclear cavity, the chromatin contributed by the respective parents remaining distinct throughout the preparation for the first division. The writer is unable to state whether fusion in the sense of complete intermingling ever occurs after the completion of the first division.

Summary

1. After the male nucleus and two polar nuclei come together, the separating nuclear membranes persist more or less until segmentation.

2. Three distinct spirems are formed in the primary endosperm nucleus.

3. A tripolar spindle, each arm with its group of chromosomes, precedes the formation of the bipolar spindle.

4. The three groups of chromosomes maintain their identity, at least until several divisions have occurred.

5. The nuclear membranes separating the egg and sperm nuclei disappear earlier than in the preceding case, but the two groups of chromatin remain separate.

6. Two distinct spirems, followed by two groups of chromosomes, arise from the maternal and paternal chromatin in the fertilized egg.

7. There is no complete intermingling of chromatin at fertilization.

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LITERATURE CITED

1. BLACKMAN, V. H., and WELSFORD, E. J., Fertilization in *Lilium*. Ann. Botany **27**:111–115. 1913.
2. COULTER, JOHN M., The endosperm of angiosperms. BOT. GAZ. **52**:380–386. 1911.
3. ERNST, A., Chromosomen reduction, Entwickelung des Embryosackes, und Befruchtung bei *Paris quadrifolia* und *Trillium grandiflorum*. Flora **91**:1–36. *pls. 1–6*. 1902.
4. FERGUSON, M. C., The development of the egg and the fertilization in *Pinus Strobus*. Ann. Botany **15**:435–479. 1901.
5. ———, Contributions to the knowledge of the life history of *Pinus*, etc. Proc. Wash. Acad. Sci. **6**:1–202. *pls. 1–24*. 1904.
6. GUIGNARD, L., Nouvelles études sur la fécondation. Ann. Sci. Nat. Bot. VII. **14**:163–296. 1891.
7. ———, Sur les antherozöides et la double copulation sexuelle chez les végétaux Angiospermes. Compt. Rend. **128**:864–871. 1899.
8. ———, Nouvelles recherches sur la double fécondation chez les végétaux Angiospermes. Compt. Rend. **131**:153–160. 1900.
9. ———, L'appareil sexuel et la double fécondation dans les *Tulipa*. Ann. Sci. Nat. Bot. VIII. **11**:365–387. 1900.
10. ———, La double fécondation chez les *Cruciféres*. Jour. Botanique **16**:361–368. 1902.
11. HEATLEY, MARGARET, A study of the life history of *Trillium cernuum* L. BOT. GAZ. **61**:425–430. 1916.

12. HUTCHINSON, A. H., Fertilization in *Abies balsamea*. BOT. GAZ. **60**:457–473. 1915.
13. MOTTIER, D. M., Über das Verhalten der Kerne bei der Entwickelung des Embryosackes und die Vergänge bei der Befruchtung. Jahrb. Wiss. Bot. **31**:125–158. 1898.
14. ———, Fecundation in plants. Carnegie Inst. Publ. **15**. 1904.
15. NAWASCHIN, S., Neuen Beobachtungen über Befruchtung bei *Fritillaria tenella* und *Lilium Martagon*. Bot. Centralbl. **77**:62. 1899.
16. SAX, KARL, Fertilization in *Fritillaria pudica*. Bull. Torr. Bot. Club **43**:505–523. 1916.
17. STRASBURGER, E., Einige Bemerkungen zur Frage nach der döppelten Befruchtung bei den Angiosperms. Bot. Zeit. **58**:293–316. 1900.
18. ———, Über Befruchtung. Bot. Zeit. **59**:1–8. 1901.

EXPLANATION OF PLATES III–V

All figures were drawn with the aid of an Abbe camera lucida, with Bausch & Lomb 1.9 mm. oil immersion, and ocular 6. The magnification of all figures except fig. 29 is ×1500; fig. 29 ×420. The plates are reduced to two-thirds their original size.

PLATE III

FIG. 1.—Early union of egg and sperm in *Lilium Martagon*.

FIG. 2.—A little later stage in *Trillium grandiflorum*.

FIG. 3.—Early prophase, separating membranes between egg and sperm having disappeared.

FIG. 4.—A little later, but separating membranes still present.

FIG. 5.—An interrupted spirem in fertilized egg, spirem being more or less beaded.

FIG. 6.—Early spirem in fertilized egg.

FIGS. 7, 8.—Development of spirem.

FIGS. 9, 10.—Two spirems in each nucleus, line *a–b* separating chromatin contributed by egg and sperm respectively.

FIG. 11.—Contraction at time of segmentation.

FIG 12.—Later, contraction still evident; chromosomes in two groups, nuclear membrane having disappeared.

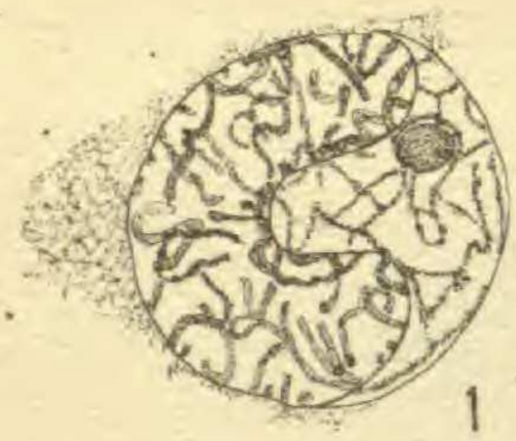
PLATE IV

FIG. 13.—Formation of bipolar spindle, second group being pulled into equatorial plate.

FIG. 14.—Late telophase of first division.

FIG. 15.—Two-celled embryo.

FIG. 16.—*Trillium grandiflorum;* early coming together of three nuclei to form primary endosperm nucleus.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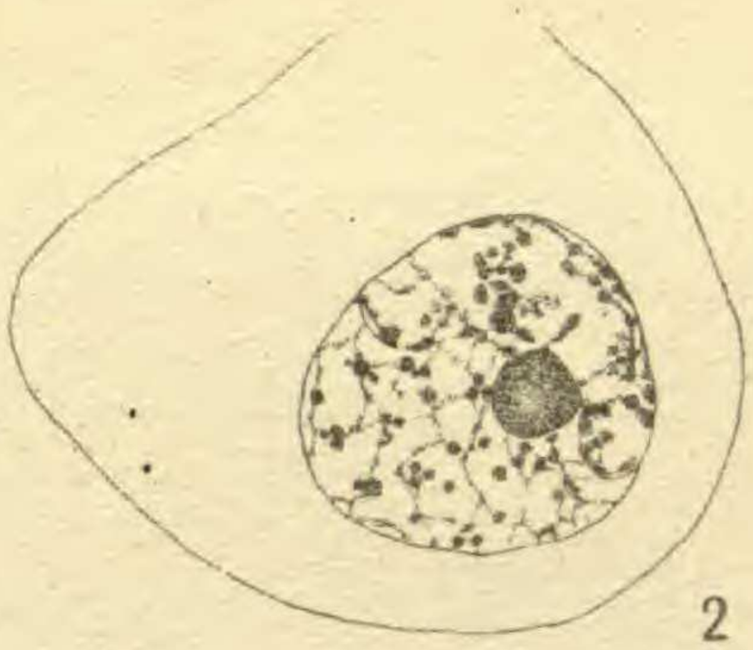
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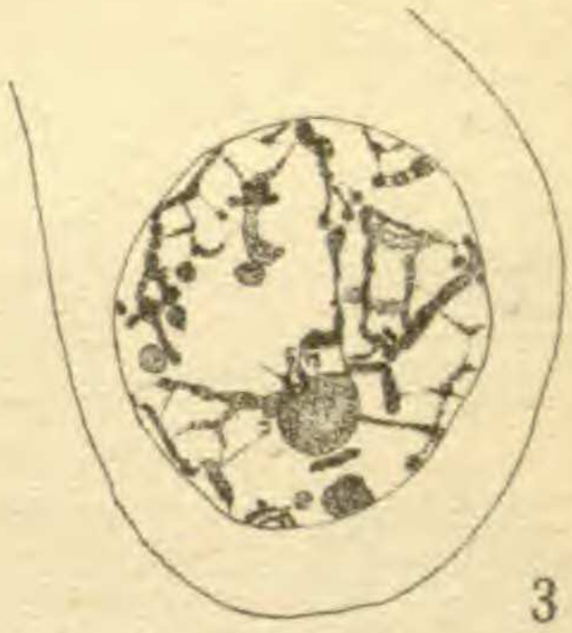
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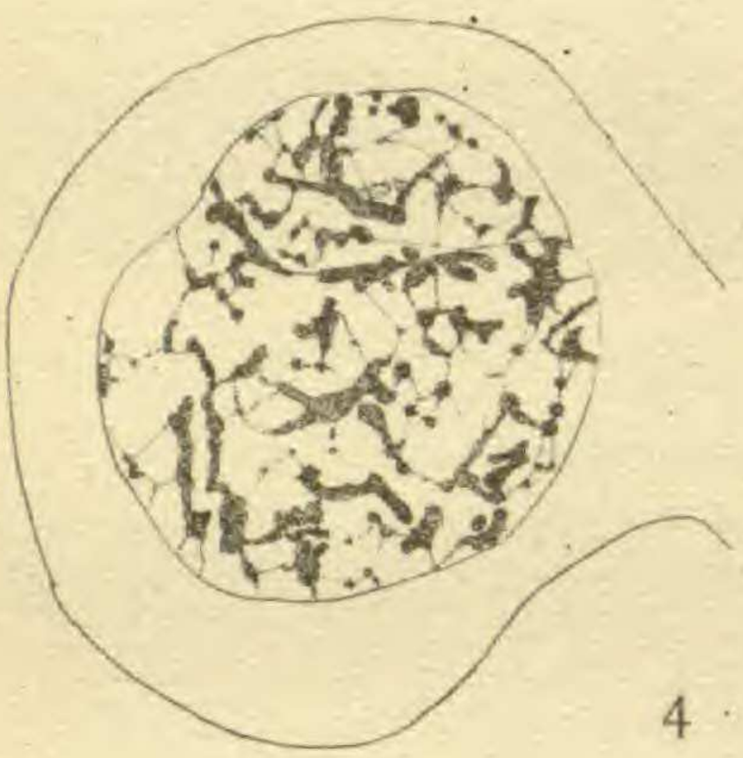
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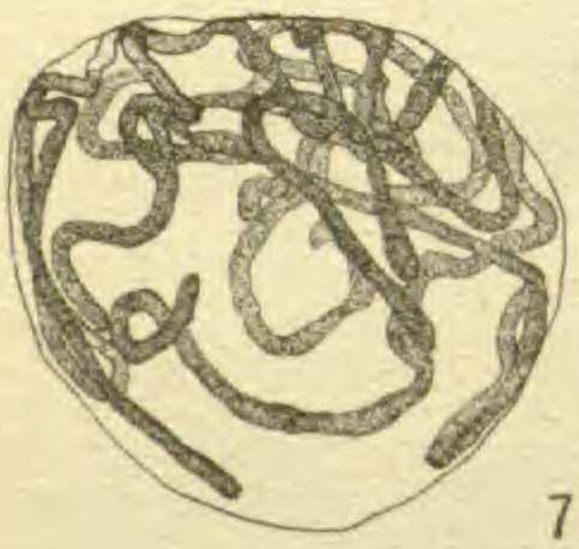
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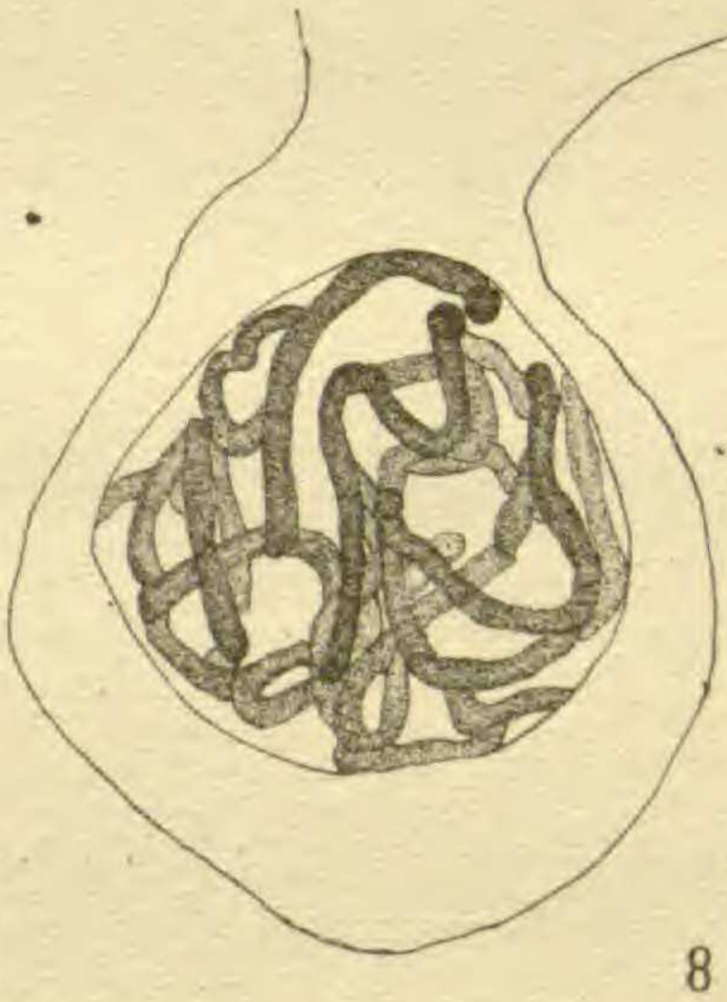
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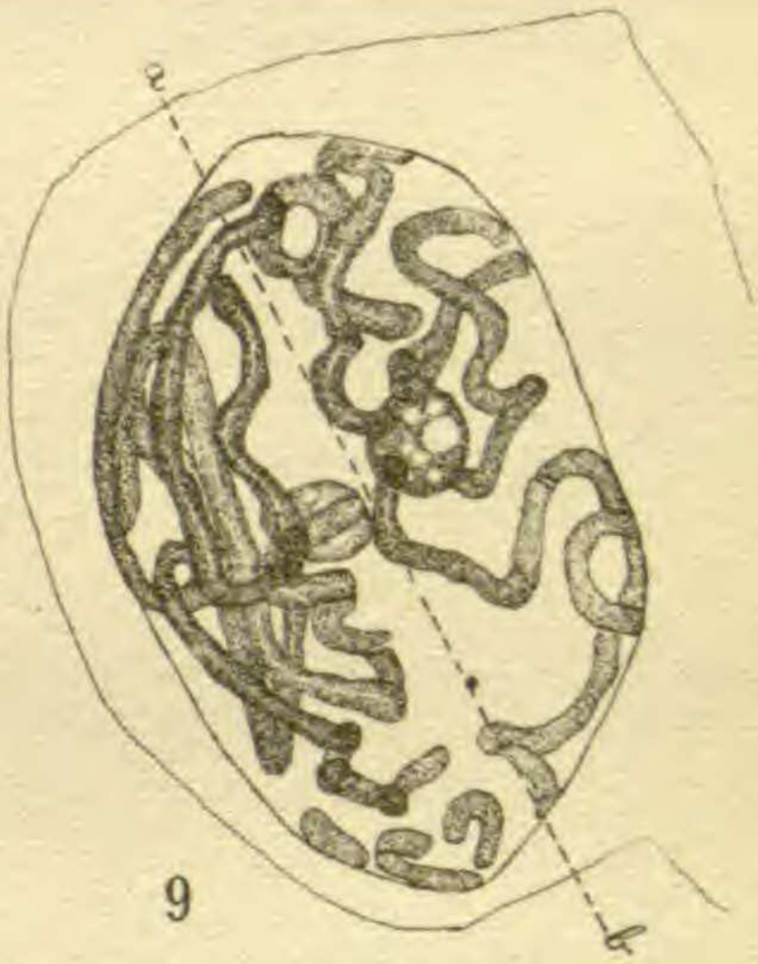
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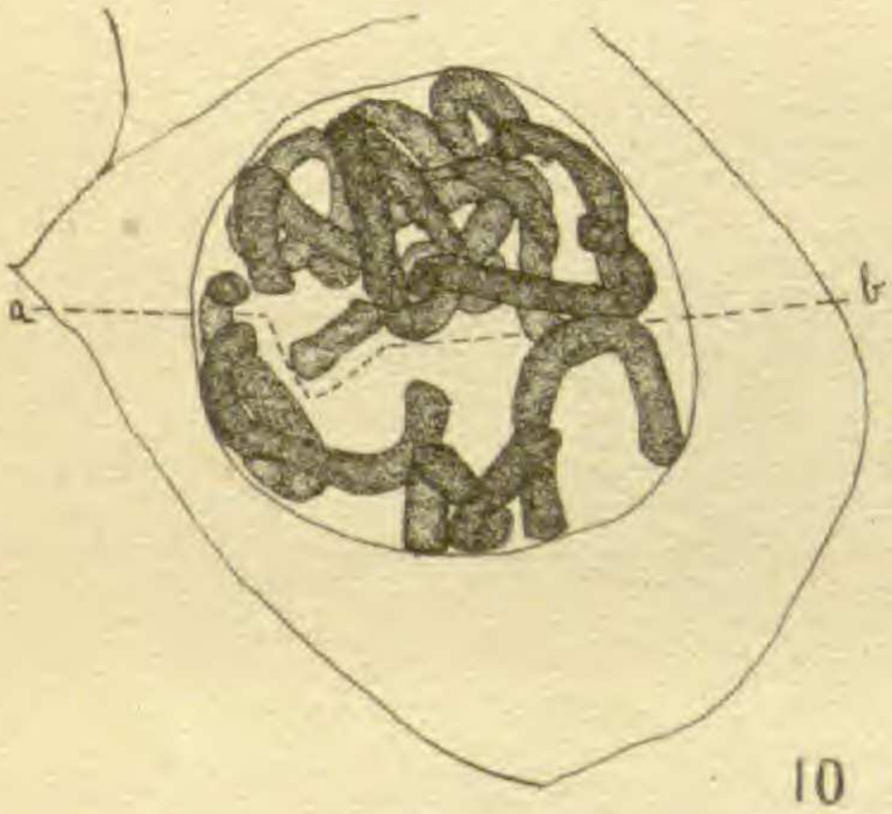

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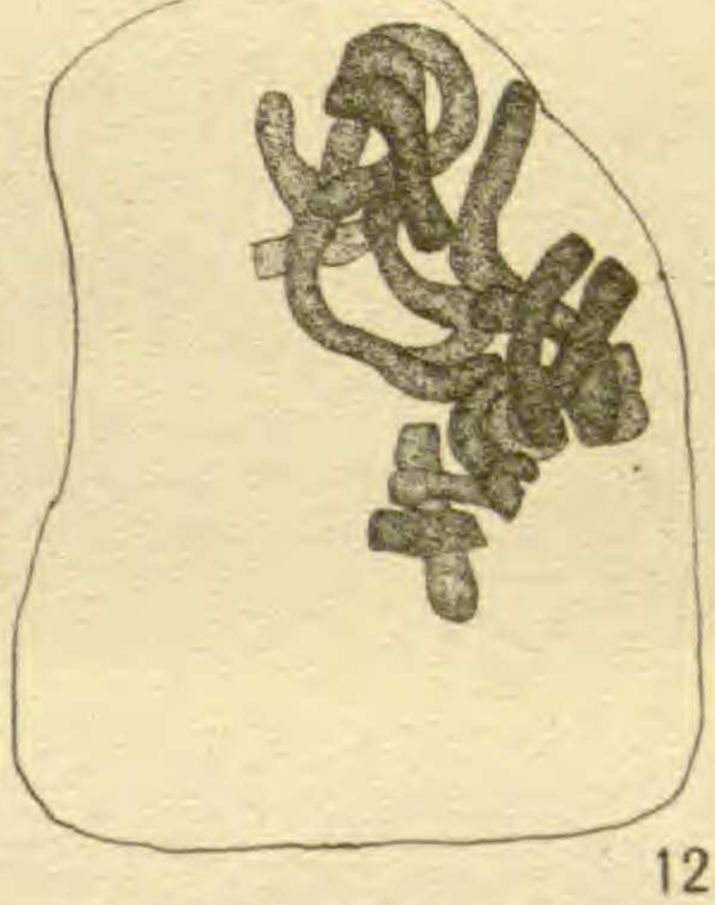
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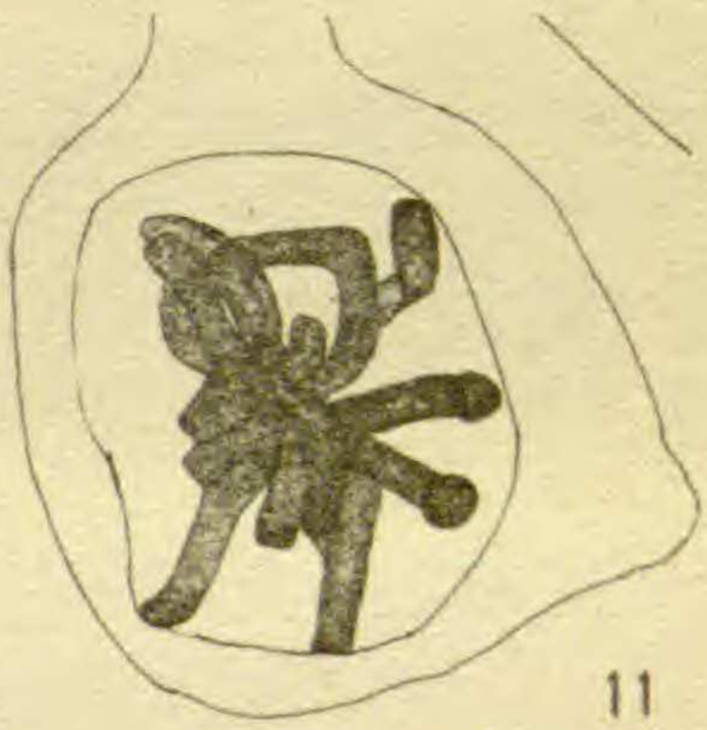
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NOTHNAGEL on FERTILIZATION